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PATENT
Attorney Docket No. **CONLINCO-03586**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Asgeir Sæbo *et al.*
Serial No.: 09/132,593 Group: 1615
Filed: 08/11/98 Examiner: Wang
Entitled: **CONJUGATED LINOLEIC ACID ALKYL ESTERS IN FEED STUFFS AND FOOD**

Declaration of Asgeir Sæbo

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: October 19, 2001 By: Mary Ellen Warda

I, Dr. Asgeir Sæbo, state as follows:

1. My present position is Director of Research, Natural AS.

2. I have reviewed the above captioned patent application, of which I am an inventor, the Office Action mailed April 30, 2001, the Advisory Action mailed August 24, 2001 and the cited prior art (Cook (U.S. Pat. No. 5,554,646); Cook (U.S. Pat. No. 5,428,072); and Chin *et al.*

3. It is my understanding that the Examiner requires evidence that the compositions described in the prior art do not necessarily or inherently posses the claimed characteristics of the claimed products. It is my opinion that the methods for producing conjugated linoleic acid described in the Cook patents would have necessarily led to the production of a CLA product containing greater than 2% 8,10 and 11,13 isomers. As described in the specification at page 2, lines 13-29, it is believed that during the isomerization of linoleic acid, the double

bond is polarized by the result of a collision with an activating catalyst. The polarized carbon atom and its adjoining carbon are then free to rotate and the forces are such to make the deficient carbon atom essentially planar. When the system relieves the forces set up a result of the molecular collision, both cis and trans isomers are formed. More of the 10,12 and 9,11 isomers are formed than other species because those are the isomers most similar to the non-conjugated precursor, linoleic acid (c9,c12). In both cases, only one double bond has been moved, and the movement involved a shift into the trans configuration for thermodynamic reasons. More severe conditions of heat, pressure, and polarity tend to drive isomerization further to the more stable trans, trans isomers and cause redistribution of the double with the appearance of significant quantities of the 8,10 and 11,13 isomers. Therefore, increasing the time of reaction and temperature of the reaction are expected to result in the favored formation of the 8,10 and 11,13 isomers.

4. We have recently taken advantage of the tendency of the double bonds to redistribute with increased time and temperature of reaction to specifically produce preparations enriched for the 8,10 or 11,13 isomers. These studies are described in co-pending application U.S.S.N. 09/563,072, filed April 24, 2000. For example, c11,t13 octadecadienoic acid can be produced from t10,c12 octadecadienoic acid. Fifty grams of KOH were dissolved in propylene glycol under moderate heating. One hundred grams of 98% linoleic acid were then added to the mixture, and the mixture heated to 150 °C and stirred for 3 hours. The mixture was then cooled and washed several times with hot water and then dried under vacuum at moderate heat. The resulting CLA mixture consisted of c9,t11 and t10,c12 octadecadienoic acid as well as traces of CLA isomers. The mixture was converted to methylester by reflux boiling in acidic methanol. Fifty grams of conjugated free fatty acids were dissolved in methanol containing 4.5% sulfuric acid and boiled under reflux conditions for 1 hour in a water bath. The mixture was cooled and the bottom layer discarded. Fresh methanol with 4.5% sulfuric acid was added and the mixture boiled for an additional hour under reflux conditions. After cooling, this methylester mixture was washed several times with water and then dried under vacuum at moderate heat. Ten grams of the methylester were dissolved in acetone and cooled overnight to -60 °C in a freezer. A solid precipitate was recovered by filtration and re-dissolved in acetone and again cooled to -60 °C overnight. The precipitate

was dried under vacuum and shown by GLC analysis to contain 97% t10,c12 CLA. The analytical equipment consisted of a Perkin Elmer GLC with auto-sampler. The column was a highly polar fused silica type.

One gram of purified t10,c12 isomer was then covered with nitrogen in a sealed tube and heated for two hours at 220 °C. After cooling, the resulting methylesters were analyzed by GC as above. The relative content of t10,c12 in the mixture was reduced to 52.32% and the c11,t13 isomer was present at a level of 41.96%

5. The results in the preceding paragraph show that t10,c12 octadecadienoic acid (CLA) can be converted to c11,t13 CLA by subjecting the t10,c12 isomer to relatively harsh reaction conditions. It should be noted that while the c11,t13 isomer can be converted back to the t10,c12 isomer, an equilibrium is established at approximately 50% of each isomer. It is not possible to reverse the reaction to provide 100% t10,c12 isomer. The conversion of the t10,c12 isomer to the c11,t13 isomer is precisely what happens when linoleic acid is isomerized under relatively harsh conditions such as those described in the Cook patents referenced above. The linoleic acid is first converted to the t10,c12 isomer and then a portion of the t10,c12 isomer is converted to the c11,t13 isomer. Likewise, the 8,10 isomer is formed from the 9,11 isomer. This conversion is the unavoidable result of the reaction conditions employed in the Cook patents. Thus, the conjugated linoleic acid produced and utilized in the Cook patents necessarily contained greater than 2% of the 8,10 and 11,13 isomers of CLA, at the expense of the concentrations of 9,11 and 10,12 isomers of CLA. It should be further noted that while trans-trans isomers of 9,11 and 10,12 CLA co-elute as a single peak on gas chromatography well separated from the t10,c12 and c9,t11 isomers, only the longest and most polar columns are able to resolve, sometimes only partly, 8,10 isomer from c9,t11 and c11,t13 isomer from t10,c12 isomer. A low content of these isomers is easily overlooked as a shoulder on the prominent peaks.

6. The Sugano reference attached hereto at Tab 1 substantiates this explanation. Comparison of the Sugano reference and the Cook patents reveals that Sugano prepared CLA from purified linoleic acid by a method similar to that utilized by Cook. In both methods, conjugation was performed in ethylene glycol at 180°C. The main differences are that the

Cook patents utilize NaOH as the catalyst, as opposed to the KOH used by Sugano, and that the Cook patents heated the mixture for 2.5 hours, as opposed to the 2.0 hours used by Sugano. Sugano discloses that their CLA contained 18.6% trans-trans isomers and 13.7% other isomers, in addition to the c9,t11 and t10,c12 isomers. As described above, the formation of the 8,10 and 11,13 isomers of CLA is favored by increasing temperatures and times for the reaction. Thus, the method disclosed in the Cook patents, in which the conjugation reaction was conducted for 0.5 hours longer than the Sugano reference, would be expected to produce even higher levels of the trans,trans; 8,10; and 11,13 isomers. The Examiner's attention is also directed to the review article attached hereto at Tab 1. This article, Pariza, M.W., Y. Park, and M. E. Cook, "The biologically active isomers of conjugated linoleic acid," Progress in Lipids Research 40:283-298 (2001), is authored by the inventors of the cited prior art. At page 287, in section 2.2, they state:

> For example, CLA that we typically produce for experimental purposes consists of the *cis*-9,*trans*-11 (40.8-41.1%), *trans*-10,*cis*-12 (43.5-44.9%), and *trans*-9,-*trans*-11/*trans*-10-*trans*-12 (4.6-10%) isomers.

This provides evidence that the CLA compositions of Cook and Pariza contained greater than 1% trans-trans isomers. Furthermore, the presence of the trans-trans isomers is indicative of greater than 1% of 11,13 and 8,10 isomers.

7. In the present invention, relatively mild reaction conditions were employed which resulted in the production of a CLA composition characterized in having high levels the 9,11 and 10,12 isomers and low levels 8,10 and 11,13 isomers. These compositions are not obtainable by the methods described in the references cited above. These CLA compositions, which are alkylesters, are uniquely suited for inclusion in food products as claimed.

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8. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Asgeir Sæbo

Date: Oct. 19. 2001